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71 Applicant(s): CLARINS LABORATOIRES S.A.S.  
FR

72 Inventor(s): COURTIN, OLIVIER

73: Proprietor(s):

74 Representative(s): BREESE MAJEROWICZ  
SIMONNOT

54 HYDRATING COSMETIC COMPOSITION CONTAINING A BOTANIC TRYPSIN INHIBITOR

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## HYDRATING COSMETIC COMPOSITION CONTAINING A BOTANIC TRYPSIN INHIBITOR

The present invention relates to cosmetic, possibly dermatological, compositions for the care of the skin, particularly to combat aging or to hydrate the skin, based on a plant extract. More particularly, the invention compositions are based on the understanding of the role of cutaneous collagen, particularly of the fact of its capacity to fix water and of the impact of its degradation during aging (C. Durandea et al., S.T.P. Pharma 5(89), 541-547 (1989)). Thus in the prior art numerous cosmetic compositions based on collagen have been proposed for their hydrating power.

The Applicant is now interested in the phenomenon of the destruction of cutaneous fibers by proteases, and more particularly of skin collagen by the collagenases (L. Robert and A.M. Robert, J. Med. Est. et Chir. Derm., Vol. XX, 80, December 1993, pp. 211-217). The cutaneous maturation of the collagenases necessitates the intervention of another proteolytic enzyme: trypsin. In fact, this protease plays a particular role in the skin where the fibroblasts synthesize numerous enzymes and collagenase, the essential function of which in the renewal of the extracellular matrix and the aging of the skin is known. Like many enzymes, it is elaborated in the form of an inactive pro-collagenase, which is cleaved by trypsin to become active.

The present invention thus has as its subject a cosmetic composition for the skin characterized by the fact that it contains a plant protein or a plant extract displaying a trypsin-inhibiting activity.

Of course, the invention envisages a cosmetically acceptable plant protein, preferably a plant extract rich in this type of protein.

A cosmetic composition in accordance with the invention is thus noteworthy in that it makes it possible to control the collagenase activity in the tissues by lowering the level of trypsin or by inhibiting it so as to block the activation cascade of the proteases of the extracellular matrix and thus to regenerate the collagen in the skin.

The invention thus offers new procedures for the cosmetic treatment of the aging or dehydration of the skin by the topical application to a subject of an efficacious amount of a botanic trypsin inhibitor or a plant extract containing such an inhibitor, and more particularly those described below.

The Applicant is more particularly interested in a class of protease inhibitors known as Bowman-Birk inhibitors. These inhibitors have already been described as displaying anti-elastic, anti-inflammatory activities. This class of inhibitors in addition display two active sites, each capable of inhibiting a protease. Thus, in the presence of a Bowman-Birk inhibitor, trypsin and chymotrypsin lose their lytic activity. The invention thus has as its subject the use of a plant trypsin inhibitor, in particular an inhibitor of the Bowman-Birk type, or a plant extract rich in one of these inhibitors as the active agent in the protection or regeneration of the skin collagen and therefore useful for the preparation of a cosmetic composition, especially an anti-aging or hydrating composition.

Among the Bowman-Birk inhibitors, the invention is aimed specifically at a plant protein called prohibine, the presence of which in several leguminous plants such as soybeans, peas and field beans, all sharing great homology of order, has been described. The invention is preferentially interested in prohibine from *Phaseolus lunatus*, the common name of which is lima bean. *Phaseolus lunatus* is widespread in Central and South America. It is a bushy plant reaching 50 cm at maturity and producing pods from which are taken beans that can be adapted to different culinary preparations. The bean is rich in protein (21%), carbohydrates (63%) and mineral salts (2.4%), but contains only very few lipids (0.6%). Prohibine is a protein synthesized by the plant, probably as a stabilizing system for the seed while awaiting germination, or is used as a defense against the attacks of insects that produce proteinases.

The present invention thus relates very specifically to a cosmetic composition containing a trypsin-inhibiting plant protein belonging to the Bowman-Birk inhibitor class or a plant extract rich in this protein. Advantageously, the invention relates to a composition containing an extract of a legume such as soybean, pea, field beans or, most preferably, *Phaseolus lunatus*. This is a protein extract or an extract rich in a protein with trypsin-inhibiting activity that can be obtained by extraction and isolation, or more or less intensive purification, by techniques known to one skilled in the art.

It has been shown within the scope of the present invention that the enzymatic activities of trypsin and chymotrypsin decrease in a dose-dependent manner, the inhibition being complete at 0.01% and already significant at 0.0005%. A cosmetic composition in accordance with the invention thus contains 0.05 to 10%, preferably 0.1 to 5% by weight of the total composition, of plant protein or of plant extract rich in this protein.

Prohibine is a protein of about 8000 daltons which has no direct enzyme activity, but its three-dimensional structure imparts to it a spatial conformation that gives it an affinity for the two proteolytic enzymes: trypsin and chymotrypsin.

Consequently, to be active, prohibine should advantageously be solubilized in a medium that permits it to preserve its conformation. The solubilization of prohibine in a medium compatible with a cosmetic formulation poses the precise difficulties that this invention proposes to resolve. In fact, prohibine is a water-soluble product that is not very compatible with surfactants, which constitute an unfavorable environment due to deformation of the tertiary structure, and lead to inactivation of the protein.

The invention relates for this reason very preferentially to a cosmetic composition containing an aqueous phase containing a plant protein or a plant extract displaying a trypsin-inhibiting activity. This can involve a lotion but, advantageously, a cosmetic composition in accordance with the invention contains two distinct phases, the said aqueous phase and an oily phase, which are mixed by shaking before use.

The oily or fatty phase makes it possible to give the invention composition more "slip" and richness. The weight ratio between the oily phase and the aqueous phase is preferably of the order of 20/80.

A composition in accordance with the invention can contain other active agents, in particular hydrating agents such as:

- an extract of algae, for example an extract of *Himanthalia elongata*, which is a brown algae of the Fucales family, the intracellular content of which is extracted and purified. This type of extract is rich in proteins, oligo-elements, mannitol and vitamins, which imparts to it hydrating properties. A composition in accordance with the invention can include of the order of 0.1 to 0.5% of this kind of extract.

- an extract of *Pinus lambertiana* or of *Pinus sylvestris*, which is a New Zealand pine of which an extract of the trunk and leaves is very rich in cyclitol (D-pinitol). This is a sugar constituting a major oligosaccharide which contributes to the survival of certain species subjected to salt stress. This sugar also has membrane-stabilizing properties which impart to this extract hydrating properties with respect to the skin. A composition in accordance with the invention can contain of the order of 0.1 to 5% of this kind of extract.

- an extract of cactus flower, more particularly of *Cereus grandiflorus*, originating in the Antilles and Central America. This is a glycolic extract of the cactus flowers which contains phenolic compounds and alkaloids that soften and hydrate the skin. A composition in accordance with the invention can contain of the order of 0.1 to 5% of this kind of extract.

- an extract of white lupin. This a protein extract of *Lupinus albus*, rich in low molecular weight glutaminated peptides and oligosaccharides. It strengthens the skin barrier by stimulating the synthesis of the lipids and proteins specific to the epidermis, thus limiting loss of water and therefore permitting better hydration of the stratum corneum. A composition in accordance with the invention can contain of the order of 0.1 to 5% of this kind of extract.

Other advantages and characteristics of the invention will appear from the examples that follow regarding the anti-trypsin and anti-chymotrypsin activity of prohibine and of compositions in accordance with the invention.

#### 1) Anti-trypsin and anti-chymotrypsin activity of prohibine

The capacity for inhibition of trypsin and chymotrypsin by prohibine was demonstrated in vitro. Two esters of substituted amines, N- $\alpha$ -benzoyl-arginine ethyl ester (BAE) for trypsin and N- $\alpha$ -benzoyltyrosine ethyl ester (BTEE) for chymotrypsin, were used as specific substrates. After hydrolysis of the ester function, the appearance of the liberated benzoylamine is followed by optical density at 256 nm.

The protein was tested at 6 different concentrations and the trypsin and chymotrypsin activities were evaluated by their capacity to liberate the specific amine.

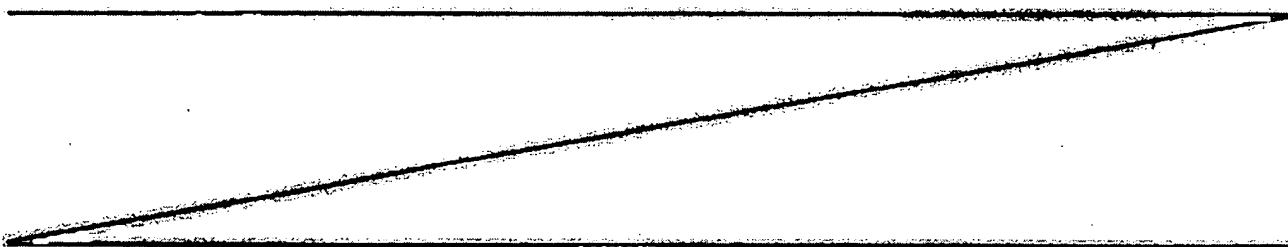
The attached Figure 1 represents the percentage hydrolysis rates in the presence of increasing concentrations of prohibine relative to the maximum hydrolysis rate of the enzymes without prohibine.

For chymotrypsin, which is the most strongly inhibited enzyme, the decrease in activity measured by the rate of hydrolysis of the amine substrate is given in Table I below.

Table I

Protein (prohibine) (%)	Chymotrypsin (%)
$5 \cdot 10^{-4}$	- 38
$1,2 \cdot 10^{-3}$	- 62
$2,5 \cdot 10^{-3}$	- 90
$5 \cdot 10^{-3}$	- 96
$10^{-2}$	- 100

#### 2) Example of a two-phase hydrating serum in accordance with the invention



<u>Aqueous phase</u>	
Purified water	QSP 100,000
Film-forming hydrating synthetic polymer	10,000
Glycerol	10,000
Sodium PCA	9,000
Dipropylene glycol	5,000
Prohibine	1,000
Himanthalia elongata	2,000
Cactus flower extract	1,000
White lupin extract	1,000
Pinus sylvestris extract	1,000
Triethanolamine	0.300
Sodium chloride	0.300
Xanthan gum	0.200
Preservatives	0.500
Sequestrant	0.100
Colorant solution	0.200
<u>Oily phase</u>	
Perhydrosqualene	40,000
Isohexadecane	37,000
Fatty acid ester	20,000
Perfume	1,800
Vitamin E derivative	1,000
Antioxidant	0.200

## CLAIMS

1. Cosmetic composition for the skin, characterized by the fact that it contains a plant protein or a plant extract displaying a trypsin-inhibiting activity.
2. Cosmetic composition as in claim 1, characterized by the fact that it contains a trypsin-inhibiting plant protein belonging to the class of Bowman-Birk inhibitors, or a plant extract rich in this protein.
3. Cosmetic composition as in one of the claims 1 or 2, characterized by the fact that it contains a protein or extract from a legume such as soybeans, peas, field beans, or *Phaseolus lunatus*.
4. Cosmetic composition as in one of the claims 1 to 3, characterized by the fact that it contains a protein from *Phaseolus lunatus* of molecular weight about 8000 displaying an inhibiting activity for trypsin and chymotrypsin, or a protein extract of *Phaseolus lunatus* containing this protein.
5. Cosmetic composition as in one of the claims 1 to 3, characterized by the fact that it contains 0.05 to 10%, preferably from 0.1 to 5%, by weight of the total composition, of a plant protein or a plant extract rich in this protein.
6. Cosmetic composition as in any one of the preceding claims, characterized by the fact that it contains an aqueous phase containing a plant protein or a plant extract displaying a trypsin-inhibiting activity.
7. Cosmetic composition as in any one of the preceding claims, characterized by the fact that it contains two distinct phases, an aqueous phase and an oily phase, the said aqueous phase containing a plant protein or a plant extract displaying a trypsin-inhibiting activity.
8. Cosmetic composition as in any one of the preceding claims, characterized by the fact that it contains one or more active agents chosen from among: an extract of algae, for example an extract of *Himanthalia elongata*, an extract of *Pinus lambertiana* or of *Pinus sylvestris*, an extract of cactus flower, or a extract of white lupin.
9. Use of a plant trypsin inhibitor or a plant extract containing such an inhibitor as an active agent in the protection or regeneration of the collagen of the skin.
10. Procedure for cosmetic treatment of the aging or dehydration of the skin by topical application in a subject of an efficacious quantity of a botanic trypsin inhibitor or of a plant extract containing such an inhibitor.



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